Theor Appl Genet (2000) 100:1290–1294

R.K. Varshney · M. Prasad · J.K. Roy · N. Kumar
Harjit-Singh · H.S. Dhaliwal · H.S. Balyan · P.K. Gupta

Identification of eight chromosomes and a microsatellite marker on 1AS associated with QTL for grain weight in bread wheat

Received: 15 September 1999 / Accepted: 9 November 1999

Abstract The present study in bread wheat was undertaken, *firstly,* to identify chromosomes carrying QTLs controlling 1000 grain weight (GW) and, *secondly,* to develop molecular marker(s) linked with this trait. Using the genotype Rye Selection111 (RS111), we carried out a monosomic analysis that suggested that 8 chromosomes (1A, 1D, 2B, 4B, 5B, 6B, 7A and 7D) carried QTLs controlling GW, with only 3 of these (1A, 2B, 7A) carrying alleles for high GW. To tag the QTLs present on these chromosomes, we crossed the genotype RS111 with high GW (56.83 g) with the genotype Chinese Spring (CS) with low GW (23.74 g) and obtained 100 RILs. These RILs showed normal distribution for GW. The parental genotypes were analysed with as many as 346 STMS primer pairs for detection of polymorphism. Of these, 267 primer pairs gave scorable amplification products, 63 of which detected polymorphism between the parents. Using each of these 63 primer pairs, we carried out bulked segregant analysis on RILs representing two extremes of the distribution. One primer pair (WMC333) showed an association of the marker locus *Xwmc333* with grain weight. This was confirmed through selective genotyping, and the co-segregation data on molecular marker locus *Xwmc333* and GW were analysed following a single marker linear regression approach. Significant regression suggested linkage between *Xwmc333* and a QTL for GW. The results showed that the above QTL accounted for 15.09% of the variation for GW between the parents. The marker has been located on chromosome arm 1AS, and QTL was designated *QGw1.ccsu-1A.*

Communicated by P.M.A. Tigerstedt

R.K. Varshney · M. Prasad · J.K. Roy · N. Kumar · H.S. Balyan
P.K. Gupta (✉)
Molecular Biology Laboratory,
Department of Agricultural Botany, Ch. Charan Singh University,
Meerut-250004, India
Fax: +91-121-760577; 767018
e-mail: pkgupta@ndf.vsnl.net.in

Harjit-Singh · H.S. Dhaliwal
Biotechnology Centre, P.A.U., Ludhiana-141004, India

Key words Bread wheat · Grain weight · Microsatellite · Monosomic analysis · QTL analysis · STMS

Introduction

Among the various grain related traits, grain weight (GW) is one of the more important ones, since it is phenotypically the most stable component of yield and is also positively correlated with flour yield. Grain weight has been shown to be controlled by a number of quantitative trait loci (QTLs) located on different chromosomes (Halloran 1976; Bannier 1979; Khrabrova and Maistrenko 1980; Chojecki et al. 1983; Zheng et al. 1993; Giura and Saulescu 1996). Unfortunately, an improvement in GW through plant breeding has generally been found to be of no consequence, since an increase in GW is known to be associated with a reduction in grain number per spike, thus neutralizing the benefit derived from the increased grain weight. Phenotypic selection for this trait is also laborious and time-consuming. Under these circumstances, the use of molecular markers for indirect marker-assisted selection for improvement in GW should be a convenient alternative.

With respect to wheat breeding molecular markers have already become available for a large number of economic traits (Gupta et al. 1999). In our own laboratory, a few sequence-tagged microsatelitte site (STMS) and sequence-tagged site (STS) markers for grain protein content and pre-harvest sprouting tolerance have been recently identified (Prasad et al. 1999; Roy et al. 1999). The study reported here was undertaken to identify chromosomes and to develop molecular markers associated with GW. For this purpose, two parents differing significantly in GW were selected, and a set of recombinant inbred lines (RILs) were derived from a cross between these two parents. The parent with high GW was used for monosomic analysis, and the parents together with the RILs were used for identification of molecular markers that are closely associated with QTLs for GW. A variety of available markers and techniques including ran-

dom amplified polymorphic DNAs (RAPDs), DNA amplification fingerprinting (DAF), microsatellite-primed polymerase chain reaction (MP-PCR), STSs and STMSs, were initially used with the parents of the RILs to detect polymorphism. Our preliminary results showed that among all of the above classes of molecular markers, PCR-based STMS and STS markers were most promising (Balyan et al. 1998). Therefore, these two types of PCR-based markers were subsequently used for the detection of polymorphism between the two parents and between the two bulks of RILs representing high and low grain weight. This led to the identification of a STMS marker, *Xwmc333*, which showed an association with GW and was found to be present on chromosome arm 1AS.

Materials and methods

Plant material

Two bread wheat genotypes differing for GW, namely Rye Selection111 (RS111) and Chinese Spring (CS), and a set of 100 RILs derived from a cross between these parents (RS111 × CS) were used in the present study. RS111 has significantly higher (more than double) 1000 grain weight (56.83 g) than CS (23.74 g). The RIL population was developed following the single-seed descent (SSD) method at Punjab Agricultural University (PAU), Ludhiana.

Evaluation of parents and RILs for grain size

The parents along with RILs were raised at PAU, Ludhiana. The data on 1000 grain weight of the parents and the RILs were scored by recording 1000 grain weight (g) from first two tillers of 5 plants from each parent /progeny.

Monosomic analysis

Monosomic analysis was carried out for GW by crossing RS111 with the euploid CS and with each of the 21 monosomics in a CS background. The comparison of mean GW of the euploid F_2 population (derived from CS × RS111) with mean GW of each of the F_2 populations (derived from CS monosomics × RS111 crosses) was carried out to identify chromosomes on which QTLs for GW may be located.

DNA isolation and STMS primers

DNA was extracted from 10- to 15-day-old seedlings raised in a growth chamber using a modified CTAB method (Weising et al. 1995). A set of 346 STMS primers were made available to us as a member of Wheat Microsatellite Consortium (WMC) under an international collaborative project. These STMS primers were designed using sequence data of clones containing microsatellites. The genomic clones were isolated from a microsatellite rich library (Edwards et al. 1996) and were sequenced by members of WMC.

Polymerase chain reaction

DNA amplifications were carried out in 25-µl reaction mixtures, each containing 100 ng template DNA, 2 µ*M* STMS primer, 200 µ*M* each of the dNTPs, 2.5 m*M* $MgCl_2$, 1× PCR buffer and 2 U Stoffel fragment (Perkin Elmer) using the following PCR profile in a Perkin Elmer DNA Thermal Cycler. Initial denaturation at 95°C for 5 min; followed by 40 cycles of 95°C for 1 min, 51°C/61°C for 1 min, 72°C for 1 min, with a ramp at the rate of 0.5°C/s; and final extension at 72°C for 5 min. The amplification products were resolved on 10% polyacrylamide denaturing gels following silver staining (Tegelstrom 1992).

Evaluation of fragment patterns

WMC333 primers were developed by Peder Weibull, Svalov (Sweden) and the sequences of these primers are available with him (peder.weibull@swseed.se). The two alleles of the molecular marker locus *Xwmc*333 were designated as *hg* (high grain weight) and *lg* (low grain weight), so that the genotypes of the RILs were classified as *hghg* or *lglg* on the basis of patterns observed in the parental genotypes (*hghg*= RS111; *lglg* = CS).

QTL analysis

Single-marker QTL analysis using linear regression was conducted following Tinker (1996). The marker allele *hg* was coded 1 and the allele *lg* was coded 0 for regression analysis.

Assignment of WMC333 to a chromosome arm

Following the conditions described above, PCR amplification with WMC333 primers was carried out initially with a set of all the 21 nulli-tetrasomic lines and, subsequently, with ditelocentrics 1AL and 1AS.

Results and discussion

Monosomic analysis

Monosomic analysis was conducted to identify individual specific chromosomes carrying QTLs for GW, which accounted for the difference in GW between RS111 (high GW) and CS (low GW). For this purpose, a comparison of mean GW of the F_2 population (CS × RS111) involving euploid parents was made with the mean GW of each of the 21 F_2 populations (CS monosomics × RS111), each involving a monosomic parent. The results indicated that the mean values of GW of 8 of the 21 F_2 populations derived from monosomics × RS111 crosses deviated significantly from the mean value of GW of the euploid F_2 population (derived from CS × RS111), suggesting that QTLs having different alleles for GW in RS111 and CS are located on 8 different chromosomes (1A, 1D, 2B, 4B, 5B, 6B, 7A and 7D). In RS111, 3 (1A, 2B, 7A) of these 8 chromosomes carried alleles for high grain weight, and the remaining 5 (1D, 4B, 5B, 6B, 7D) carried alleles for low grain weight (Table 1). A number of previous studies provide evidence for the distribution of loci controlling GW on almost all of the wheat chromosomes (Kuspira and Unrau 1957; Law 1967; Halloran 1976; Bannier 1979; Khrabrova and Maistrenko 1980; Chojecki et al. 1983; Snape et al. 1985; Zheng et al. 1993; Giura and Saulescu 1996). Apparently, therefore, the two parents used in the present study had similar alleles for QTLs on 13 of the 21 chromosomes in the haploid complement. Therefore,

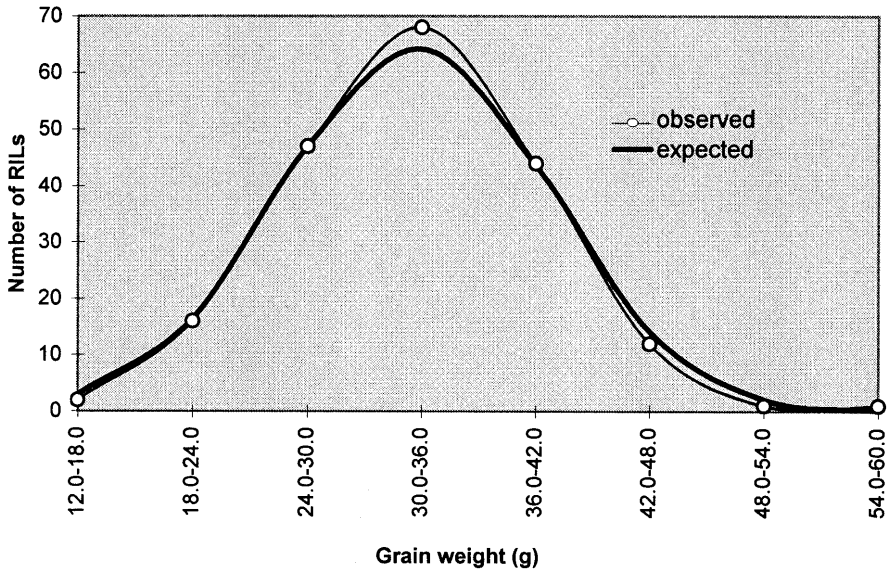

Fig. 1 Frequency distribution of grain weight in RILs showing a good fit to the normal distribution

Table 1 Grain weight (GW) in parents, F_1 and F_2 of the cross CS × RS111 and the comparison of the F_2 mean with the F_2s developed from individual F_1 monosomics

Parents and populations	Number of plants	1000 grain weight	*t* value
CS	10	28.58 ± 0.47	
RS111	10	62.19 ± 0.59	
CS × RS111(F_1)	10	44.76 ± 0.59	
CS × RS111 (F_2)	40	36.37 ± 1.05	
Mono1A × RS111	40	41.51 ± 0.82	3.81*
Mono2A × RS111	40	38.59 ± 1.03	1.69
Mono3A × RS111	40	38.60 ± 1.05	1.48
Mono4A × RS111	40	38.40 ± 1.35	1.17
Mono5A × RS111	40	37.09 ± 1.32	0.51
Mono6A × RS111	40	37.64 ± 0.96	0.88
Mono7A × RS111	31	41.10 ± 1.38	2.74*
Mono1B × RS111	40	34.56 ± 1.07	1.19
Mono2B × RS111	40	39.52 ± 1.09	2.04*
Mono3B × RS111	32	35.41 ± 1.37	0.56
Mono4B × RS111	31	32.41 ± 1.28	2.19*
Mono5B × RS111	40	32.27 ± 1.27	2.45*
Mono6B × RS111	40	28.69 ± 1.01	5.18*
Mono7B × RS111	40	36.20 ± 0.91	0.12
Mono1D × RS111	40	33.02 ± 1.14	2.13*
Mono2D × RS111	39	35.56 ± 0.95	0.56
Mono3D × RS111	40	36.70 ± 1.03	0.22
Mono4D × RS111	40	34.88 ± 1.33	0.87
Mono5D × RS111	40	35.81 ± 0.89	0.40
Mono6D × RS111	40	34.66 ± 1.08	1.13
Mono7D × RS111	40	30.90 ± 1.09	3.57*

[a] Significant (at 5% level) deviation of GW of monosomic F_2s from GW of euploid F_2 population

while making use of the above two parents for developing molecular markers for GW, one may restrict furhter analyses to only the above 8 chromosomes if chromosome-specific markers are available for developing molecular markers associated with GW. This does not, however, preclude the possibility of developing molecular markers located on other chromosomes if another pair of parents is used for developing RILs.

Grain weight in recombinant inbred lines

One hundred RILs were developed from the cross RS111 × CS following single seed descent. The RILs were raised at PAU, Ludhiana, and the resultant data on GW were scored. The 1000 grain weight (56.83 g) of the RS111 parent differed significantly from the 1000 grain weight (23.74 g) of the CS parent. The 1000 grain weight of the RILs ranged from 17.78 g to 53.38 g. Using this data on GW of RILs, we prepared a frequency distribution curve (Fig. 1); a chi-square test suggested a good fit ($P > 0.60$) to the normal distribution, indicating that the two parents differed at several QTLs controlling this trait. Our results are in conformity with the results of several earlier studies, where many loci located on several different chromosomes were reported to control this trait (Halloran 1976; Bannier 1979; Khrabrova and Maistrenko 1980; Chojecki et al. 1983; Zheng et al. 1993; Giura and Saulescu 1996; Campbell et al. 1999).

Marker identification

A total of 346 STMS primer pairs were used for the detection of polymorphism between the two parental genotypes. Of the above primers, 267 (77%) primers proved to be functional in giving scorable amplification products. Sixty-three (24%) of these primers detected reproducible polymorphism between the parental genotypes. Using these 63 primers, we conducted bulked segregant analysis (BSA, Michelmore et al. 1991) on two pooled DNA samples, each consisting of 8 RILs, representing the two tails of the normal distribution. Only the WMC333 primer pair exhibited amplification profiles characteristic of high- and low-GW parents in the corresponding bulks following BSA (Fig. 2). This suggested an association of this marker with one or more QTLs for

GW. This association was confirmed by selective genotyping (Lander and Botstein 1989) of individual RILs belonging to the two bulks (Fig. 3). The results revealed that each of the 8 RILs belonging to the high-GW pool showed a profile similar to that of the parent with high GW, while out of 8 RILs belonging to the low-GW pool, as many as 6 RILs gave a profile similar to that of the parent with low GW. This initially suggested a very strong association between the WMC333 marker and GW (the association was subsequently found not to be so strong; see following). All 100 RILs were then genotyped using the above STMS primer pair, and the data on segregation of the marker were recorded for conducting QTL analysis.

Assignment of WMC333 to chromosome arm 1AS

The microsatellite locus, *Xwmc333* (174 bp), was amplified in all nulli-tetrasomic lines, except the one that was nullisomic for 1A. Further, the amplification product was obtained using template DNA of ditelocentric 1AS and not that of 1AL, suggesting the presence of the *Xwmc333* microsatellite locus on chromosome arm 1AS.

QTL analysis

Since the data on GW of RILs fit with a normal distribution (Fig. 1), suggesting the involvement of many QTLs, QTL analysis using the single-marker linear regression approach was conducted on co-segregating data of RILs involving GW and *Xwmc333* (Tinker 1996). The regression of GW on the marker locus *Xwmc333* was highly significant (Table 2), indicating a linkage between the marker locus and the QTL for GW (designated as *QGw1.ccsu-1A*). The R^2 value of 0.1509 suggested that the marker-linked QTL contributed 15.09% of the total variation in grain weight of RILs (Fig. 4). Since the above QTL controls only a part of the total variation and the RILs showed a good fit to a normal distribution,

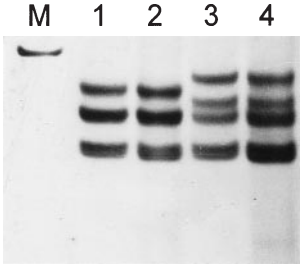

Fig. 2 Bulked segregant analysis of RILs (representing extreme groups) with WMC333 primers. *Lane M* 100-bp ladder marker; *2,3* parents, CS and RS111; *1,4* bulked segregants for low and high grain weight

Table 2 Regression analysis of grain weight on *Xwmc333* STMS marker locus

Source	Degrees of freedom	Mean square	F value	*P* value
Regression	1	3.76649	17.41647	<0.01
Residual	98	0.21626		
Total	99			

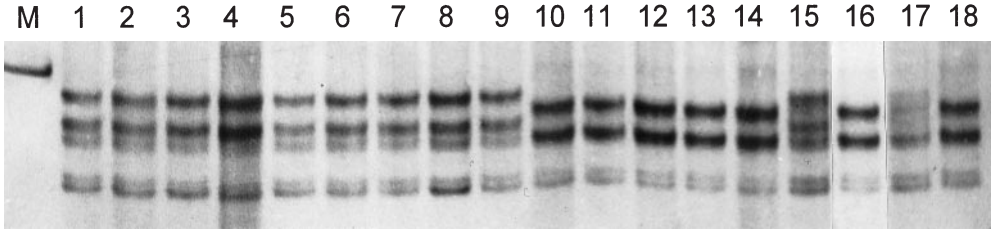

Fig. 3 Selective genotyping of RILs (representing extreme groups) with WMC333 primers. *Lane M* 100-bp ladder marker; *1,10* parents, RS111 and CS; *2–9* RILs with high grain weight; *11–18* RILs with low grain weight

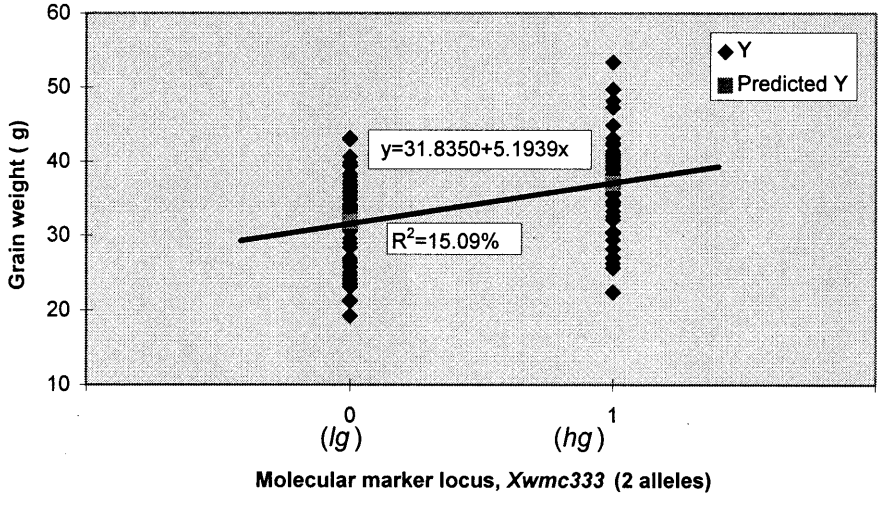

Fig. 4 Regression of grain weight (*Y*) on molecular marker locus (*Xwmc333*), drawn using single-marker linear regression QTL analysis. *lg* low grain weight, *hg* high grain weight

there will certainly be other QTLs controlling the difference in GW between the parents. Such a presence of many QTLs for GW was also suggested in a recent study, where using RFLP markers, five QTLs for 1000 kernel weight were shown to control 44% of the phenotypic variation (Campbell et al. 1999).

In view of the above, it should be possible to identify additional QTLs for GW in the future using chromosome-specific markers for the 8 chromosomes that were identified during the present study through monosomic analysis. The remaining QTLs for GW on these 8 chromosomes perhaps control a substantial proportion of the remaining variation for GW between the two parents used in the present study, but in other genotypes with high GW, additional chromosomes may be involved. Chromosome-specific RFLP and STMS markers are now available for all wheat chromosomes and should be used, if chromosomes carrying QTLs contributing to differences in GW are known. Since RFLP markers in a polyploid crop like bread wheat are relatively unsuitable for maker-assisted breeding, additional PCR-based markers (STMS, STS) should be developed to make marker-assisted breeding for GW really effective. Using the approach of functional genomics, expressed sequence tags (ESTs) may also be identified. Meanwhile, the marker developed during the present study can certainly be used for marker validation followed by its use in actual marker-assisted selection in breeding for GW.

Acknowledgements We thank M. Röder, IPK, Gatersleban, Germany for supplying the DNA aliquots of nulli-tetrasomic lines; A.J. Worland, JIC, Norwich, UK for supplying the seed material of ditelocentric line. The WMC333 clone was sequenced at Svalöf Weibull AB, Svalöv, Sweden and the primers were designed through the efforts of an international Wheat Microsatellite Consortium (WMC). The experiments described in this manuscript comply with the current laws of the country. We gratefully acknowledge financial support from the Department of Biotechnology, Government of India. Thanks are also due to the Council for Scientific and Industrial Research, Government of India for providing a CSIR-ES to PKG and a CSIR-SRF to RKV,

References

Balyan HS, Sharma PC, Ramesh B, Kumar A, Varshney RK, Roy JK, Dhaliwal HS, Singh H, Gupta PK (1998) Towards development of molecular markers for tagging genes for quality traits in bread wheat. In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symp, vol. 4. University Extension Press, University of Saskatchewan, Saskatoon, Sask., Canada, pp 84–88

Bannier E (1979) Monosomic analysis of the inheritance of 1000 grain weight in the winter wheat varieties, Karkaz and Orlando. Arch Zuchtungsforsch 9: 209–214

Campbell KG, Bergman CJ, Gualberto DG, Anderson JA, Giroux MJ, Hareland G, Fulcher RG, Sorrells ME, Finney PL (1999) Quantitative trait loci associated with kernel traits in soft × hard wheat cross. Crop Sci 39: 1184–1195

Chojecki AJS, Gale MD, Bayliss MW (1983) Reciprocal monosomic analysis of grain size in wheat. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Maruzen, Kyoto, Japan, pp 1061–1071

Edwards KJ, Barker JHA, Daly A, Jones C, Karp A (1996) Microsatellite libraries enriched for several microsatellite sequences in plants. BioTechniques 20: 758–760

Giura A, Saulescu NN (1996) Chromosomal location of genes contolling grain size in a large grained selection of wheat (*Triticum aestivum* L.). Euphytica 89: 77–80

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their application in wheat breeding. Plant Breed 118: 369–390

Halloran GM (1976) Genetic analysis of hexaploid wheat, *Triticum aestivum* using intervarietal chromosome substitution lines- protein content and grain weight. Euphytica 25: 65–71

Khrabrova MA, Maistrenko OI (1980) Monosomic genetic analysis of caryopsis protein content and weight in bread wheat Diamant 2. Genetika 16: 1425–1434

Kuspira J, Unrau J (1957) Genetic analyses of certain characters in common wheat using whole chromosome substitution lines. Can J Plant Sci 37: 300–326

Law CN (1967) The location of genetic factors contolling a number of quantitative characters in wheat. Genetics 56: 445–461

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88: 9828–9832

Prasad MP, Varshney RK, Kumar A, Balyan HS, Sharma PC, Edwards KJ, Singh H, Dhaliwal HS, Roy JK, Gupta PK (1999) A microsatellite marker associated with a QTL for grain protein content on chomosome arm 2DL of bread wheat. Theor Appl Genet 99: 341–345

Roy JK, Prasad M, Varshney RK, Balyan HS, Blake TK, Dhaliwal HS, Singh H, Edwards KJ, Gupta PK (1999) Identification of a microsatellite on chromosome 6B and a STS on 7D of bread wheat showing association with preharvest sprouting tolerance. Theor Appl Genet 99: 336–340

Snape JW, Law CN, Parker BB, Worland AJ (1985) Genetical analysis of chromosome 5A of wheat and its influence on important agronomic characters. Theor Appl Genet 71: 518–526

Tegelstrom H (1992) Detection of mitochondrial DNA fragments. In: Hoelzel AR (ed) Molecular genetic analysis of populations: a practical approach. IRL Press, Oxford, pp 89–114

Tinker NA (1996) New strategies for QTL mapping. Barley workshop (no. 15) at the Plant Genome IV Conf. (http://www.css.orst.edu/research/barley/qtl)

Weising K, Nybom H, Wolff K, Meyer W (1995) DNA Fingerprinting in Plants and Fungi. CRC press, Baton Rouge, Fla.

Zheng YL, Yan J, Yang JL (1993) Localization of genes for grain weight in common wheat. Acta Agron Sin 19: 304–308